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BIOLOGICAL BULLETIN

THE INFLUENCE OF INBREEDING ON VIGOR IN HYDATINA SENTA.¹

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INTRODUCTION.

In some experiments dealing with the inheritance of certain egg characters in the rotifer *Hydatina senta*, it has been necessary to inbreed the animals a number of times in succession. Evidence relating to the influence of inbreeding on vigor has thus been incidentally obtained. Since this part of the evidence from the experiments has no direct bearing on the inheritance of the egg characters in question, it is published here separately.

DESCRIPTION OF THE EXPERIMENTS.

All the experiments started from a single female, herself an F₁ from a cross. From her was bred a parthenogenetic line of 12 generations. Some of the females and males of this line were paired, and a considerable number of fertilized eggs obtained. When the fertilized eggs hatched, two of the earliest hatchers, one from a family in which a large proportion of the eggs hatched,

¹ Contributions from the Zoological Laboratory of the University of Michigan No. 139.

one from a family in which only a few hatched were selected for further breeding. From these two females were bred two parthenogenetic lines, of four and ten generations, respectively. In each line some females were paired with males of the same line, and from one of the fertilized eggs of each lot a new parthenogenetic line was reared. The members of these were again inbred, and so on, six times in succession. There were thus obtained two series of parthenogenetic lines, each one after the first obtained by inbreeding from the line preceding. In one of these series, numbered I. in the table, the numbers of families in the six lines were 12, 4, 5, 17, 5, and 9, respectively; in the other series (II.) the numbers of families in successive lines were 12, 10, 8, 11, 21, and 17, respectively.

I shall attempt to show, in what follows, that there is a progressive decrease in the vigor of these six lines, from first to last.

TABLE I.

SHOWING DECREASE OF VIGOR, AS MEASURED BY VARIOUS CHARACTERS, IN SIX SUCCESSIVELY INBRED PARTHENOGENETIC LINES OF *Hydatina senta*.

Series.	Character to be Measured.	Number of Parthenogenetic Line.					
		1	2	3	4	5	6
I.	Size of family of parthenogenetic female...	48.4	42.5	46.8	42.5	31.0	22.6
	Size of family of fertilized sexual female...	16.7	12.8	12.8	11.5	6.3	7.3
	Number of eggs laid per day.....	11.0	11.4	10.3	10.0	9.2	7.5
	Number of days required to reach maturity	2.27	1.66	2.25	1.93	2.25	2.12
	Proportion of cases in which first daughter did not become parent.....	1/11	1/3	2/4	3/16	0/4	5/8
	Same in percentages.....	14.2		25.0		41.6	
II.	Size of family of parthenogenetic female...	48.4	30.8	41.0	37.0	33.8	24.8
	Size of family of fertilized sexual female...	16.7	13.7	13.5	15.2	10.1	7.6
	Number of eggs laid per day.....	11.0	11.6	7.9	7.7	9.6	8.6
	Number of days required to reach maturity	2.27	1.55	2.57	2.20	1.90	2.00
	Proportion of cases in which first daughter did not become parent.....	1/11	4/9	2/7	2/10	8/20	7/16
	Same in percentages.....	25.0		23.5		41.6	

THE MEASURE OF VIGOR.

Six distinct means of measuring the vigor of the several parthenogenetic lines are available. They are as follows:

i. Size of family of parthenogenetic females. With few exceptions every daughter of a female used for breeding was isolated and recorded. The average size of family was computed for each parthenogenetic line separately. Families not completely

recorded, of which there were a few, were not included in this computation.

2. Size of family of fertilized sexual females. It appears that the male does not appreciably affect the number of eggs laid by the female with which he is mated. The size of family is determined almost wholly by the female herself. In determining size of family in this case, I have counted the eggs laid, not the number that hatch, as experiments have led me to conclude that the vigor of the parent is responsible for the number of eggs, but not for their viability.

3. Number of eggs laid per day. The young rotifers were isolated and recorded about the same time each day. To find the average number of eggs per day in a given line, the total number of offspring hatched by that line was divided by the number of days on which they were produced, the days for each family being counted separately. Each family was produced usually in three to five days. In a line comprising nine generations, therefore, the number of days as used in this computation would be 27 to 45, regardless of the fact that several families were producing young at the same time, and that the experiment covered only about 20 days. The first day and the last day on which a given female produced daughters were counted as half days, which they must have been on the average, since the offspring recorded as of one such day must have been in some cases the output of a few hours, in other cases of practically a whole day. It is assumed that the eggs hatched in fairly uniform time, as observations have shown that they do.

4. Number of days required to reach maturity. By this is meant the interval between the laying of an egg and the time when the female that hatches from it begins to lay eggs. This time varies considerably in different lines even when reared under identical conditions. It has been found in some cases that in one line that has passed through over ninety generations parthenogenetically, about three days are required to reach maturity, while a young and vigorous line reared at the same time required less than two days. In the present experiments the time required to reach maturity is an average of the parents of all the generations in a given line. It is the interval between the hatching of the first daughter of the first generation and the hatching of

the first daughter of the last generation, divided by one less than the number of generations.

5. Proportion of cases in which the first daughter did not become the parent of the next generation. In all my breeding experiments, whenever a new family was started, the first two daughters were set aside for further breeding, though only one of them was ordinarily used. If the first daughter was apparently healthy and vigorous, she was invariably used. If the second daughter was distinctly more vigorous than the first, the second became the parent of the next generation. Sometimes it was deemed advisable to discard both and use the third, fourth, or fifth daughter. Thus, in a vigorous line, the first daughter should usually be healthy enough for breeding. As vigor decreased there should be an increasing proportion of cases in which the first daughter was replaced by a later member of the family. I was unconscious of any selection that would have favored other than the first individual in the later lines of each series, for the idea of measuring vigor by this method did not occur to me until the experiments were all finished and the data were being compiled.

6. Difficulty of rearing. As the primary purpose of the experiments was not to test vigor, but to obtain a large amount of data regarding egg characters, every effort was made to keep the conditions of nutrition, chemical composition of the medium, etc., at the optimum. To this end the food cultures were changed as frequently as seemed advantageous. If the rotifers became less vigorous, they would be more sensitive to changes in the food cultures, and it would be necessary to renew the latter more frequently.

RESULTS OF THE EXPERIMENTS.

The first five of these measures of vigor can be expressed in figures. The sixth, though not thus expressible because records were not preserved, is not less valuable. Table I. gives the data under the first five headings.

The table shows that, notwithstanding fluctuations, there is an evident decrease in the size of family, of both parthenogenetic and sexual females, from the first line of each series to the last line.

Notwithstanding fluctuations that are usually small, but occasionally large, the number of eggs laid per day is noticeably less in the later lines than in the earlier ones.

The number of days required to reach maturity remained practically unchanged throughout each series, though the temperature was higher in the later experiments than in the earlier. The first line was reared in November, the last line in June. The higher temperature in May and June should have reduced the time required to reach maturity in those months. In June of the preceding year, two lines which were the ancestors of those given in Table I., but had not been inbred, showed an average time of 1.42 days and 1.56 days, respectively, to reach maturity. The fact that the rate of growth remained practically unchanged in the inbred lines, notwithstanding the increase of temperature in the later experiments, favors the conclusion that the later lines were less vigorous.

The proportion of cases in which the first daughter of a family was not vigorous enough to become the parent of the next generation shows so much fluctuation in the six lines separately that I have combined them two by two. Although the irregularities are not thereby completely removed, it is plain that the later lines include a larger proportion of families bred from other than the first daughter than do the earlier lines.

In regard to the sixth measure of vigor, the difficulty of rearing, figures are not available because records were not preserved. It was evident at the time of the experiments, however, that the difficulty of keeping suitable food cultures gradually increased as the inbreeding proceeded. Whereas each culture was satisfactory for three or four days in the first experiments, they usually lasted less than two days at the end. This was not due to chemical changes hastened by higher temperatures in the later cultures, for cool periods in the last experiments, when the temperature was lower than the room temperature maintained in the first experiments, did not make the cultures last as long as in the earlier lines. Furthermore, that nothing was wrong with the food cultures themselves was shown by rearing rotifers from an entirely different source, presumably not often inbred, and obtaining from them healthy and vigorous families. The

rotifers of the latter experiments must have been more sensitive to adverse conditions.

With all these measures pointing in the same direction, the evidence of decrease of vigor with successive inbreeding seems conclusive.

OTHER EVIDENCE RELATING TO VIGOR IN INBREEDING.

In one of my earlier experiments (Shull, 1911), two parthenogenetic lines of *Hydatina* were crossed and a new line started from one of the F₁ fertilized eggs. The F₁ line was more vigorous, as measured by size of family and rate of growth, than was either parent line. This increased vigor in F₁, which Whitney (1912) has since shown to be general in *Hydatina*, is without doubt the same phenomenon as the decrease of vigor with inbreeding. Later experiments of my own (Shull, 1912), which involved inbreeding twice in succession, though affording some evidence of an attendant decrease of vigor, were in part contradictory. In the light of the present evidence, these contradictions appear to be merely the fluctuations, such as are found in Table I., and all that was needed to clear them up was further inbreeding. So far as *Hydatina* is concerned, there is thus entire agreement in the results of different experiments and different investigators.

In other animals and in plants there has been accumulated a large amount of information leading to the same conclusion, though not without exception, that inbreeding is attended by deterioration. On the animal side it has long been a commonplace among practical breeders that inbreeding, at least in many cases, is followed by a weakening of stock. Results of scientific value have been reported in recent years. Castle (1906) found that inbreeding the fruit-fly *Drosophila* probably reduces productiveness slightly (though this reduction could be prevented by selection). Moenkhaus (1911) inbred the same fly (*Drosophila*) and found the operation attended by a considerable increase of sterility (failure of the eggs to hatch). He was not inclined, however, to class sterility as a loss of vigor, since other attributes of vigor were not perceptibly diminished. More decidedly favoring the view that inbreeding reduces vigor is the older work of von Guaita (1898) on the mouse and Ritzema Bos (1894) on rats.

The great stature of plant hybrids was noted by Kölreuter (1763); numerous examples were cited by Gärtner (1849); and experiments with many plants were recorded by Darwin (1876). The value of crossing was known to Beal (1876) who made recommendations in regard to the rearing of corn and other plants of commercial value. More recently there have been a number of discussions, accompanying new evidence from plants. Maize has shown in the hands of G. H. Shull (1908), East (1908), and Collins (1910) that inbreeding is accompanied by deterioration, and that crossing between distinct lines brings about an increase of vigor in F_1 . East and Hayes (1912) obtain similar results in some crosses of tobacco, though not in all, and Wellington (1912) finds that the yield of tomatoes is increased by hybridization.

Further citation of such evidence would be superfluous, as rather full bibliographies have been given in recent papers, notably that of East and Hayes (1912).

MENDELIAN EXPLANATIONS OF VIGOR.

To explain the cases in which inbreeding is accompanied by deterioration, several theories have been advanced in recent years. Some have suggested that inbreeding greatly increases the chance of producing pure recessive combinations; it is necessary to assume also that these recessive characters are bad. But others have pointed out that there is equal chance that individuals homozygous for good qualities may be produced.

Two other Mendelian explanations have been offered. One was proposed by G. H. Shull (1908) to explain the greater vigor of F_1 plants of common maize. He found that successive self-fertilization in corn reduced vigor rather rapidly at first and more slowly afterwards, while crossing two unrelated lines resulted in much more vigorous plants in F_1 . He attributed the vigor of F_1 to its "hybridity," and the gradual reduction of vigor with self-fertilization to the gradual establishment of the homozygous condition. Whether there is supposed to be a special set of genes for vigor, or whether heterozygosity in ordinary body characters is held responsible for vigor, Shull does not state. Presumably the genes are not all equipotent, so that heterozygosity in one character may contribute more to vigor than heterozygosity in

another character. A similar view is advocated by East and Hayes (1912), but these authors specify that heterozygosis is responsible for only part of the vigor of an individual. The remainder they speak of as "inherent natural vigor" and leave it unexplained.

The other Mendelian explanation is that of Bruce¹ (1910). According to Bruce's view, there is an indefinite number of genes concerned with each element of vigor, for example, size. Each element of vigor depends on the number of genes present, but dominance is complete, or nearly complete, so that $MmNn$ contributes as much, or nearly as much, to vigor as does $MMNN$. All these genes are held to be equipotent so that $MmNn$ contributes twice as much to vigor as MM , and just as much as $XxYy$. Vigor is therefore proportional, on this view, to the number of different kinds of gene present, whether in homozygous or heterozygous condition. Essentially the same explanation—the bringing together of dominant characters in the zygote, some of which existed in one parent, others in the other parent—was later offered by Keeble and Pellew (1910) to explain greater stature in F_1 hybrids of certain peas. This would, of course, produce heterozygosis in these characters, but it was the presence of the genes, not their heterozygous condition, to which the authors appealed as an explanation.

As between these last two Mendelian views, the evidence does not now decide; but if either one is correct, the other can, with sufficient work, be proven incorrect.

On Shull's view, according to which vigor depends on the number of genes for which the individual is heterozygous, although a single inbreeding of a heterozygous line or a single selfing of a heterozygous individual might, in a few cases, produce offspring heterozygous in just as many genes, and therefore just as vigorous, as its parents; yet successive inbreeding or selfing must, by the laws of chance, eventually result in pure homozygous individuals (homozygous for presence or absence it matters not which). Thus in every pure line (which by definition is homozygous) the minimum of vigor has been reached, and that mini-

¹ This statement of Professor Bruce's view is taken in part from his paper, in part from correspondence with the author.

mum must be the same¹ for every pure line. Inbreeding must, on this view, always eventually reduce vigor if there is random segregation and recombination.

On Bruce's view, according to which vigor depends on the number of different kinds of gene present, there ought to be some cases in which the inbreeding of a heterozygous line or the selfing of a heterozygous individual would result in F_1 , or F_2 , or F_3 , etc., having as many present genes as the parent. Thus a parent having the constitution $AaBbCcDd$ might, on selfing for one or more generations, have a few progeny with the formula $AABB-CCDD$. Such individuals should be as vigorous as the original parent, or, if dominance is not complete, even more vigorous; and their progeny, produced by self-fertilization, should never show decrease of vigor. Any individuals that came to have the constitution $AABBCCdd$ would be a little less vigorous. Those having the formulas $AABBccdd$ and $AAbbccdd$ would be still less vigorous, and the constitution $aabbccdd$ would represent the minimum of vigor. Thus, if four genes were concerned with vigor, it should be possible to isolate four pure lines, each with its own degree of vigor, which would not thereafter decrease. Each line reaches a minimum of vigor, but that minimum is not the same for different lines.

Either of these two views, as stated, fits the evidence so far obtained from cases in which inbreeding reduces vigor, though evidence could be obtained, at the expense of sufficient labor, which would not fit both. East and Hayes's addition to the heterozygosis view, which would probably be assented to by Shull, namely, the postulation of an "inherent vigor" not dependent on heterozygosis, would make it possible to produce pure lines having different degrees of irreducible vigor. But in no case could a pure line, derived by successive inbreeding from an F_1 that was more vigorous than its parents, be as vigorous, on the view of Shull, or of East and Hayes, as the original F_1 ; this would be possible, as explained above, on the view of Bruce. If many genes were concerned with vigor, testing the correctness of the two hypotheses on the basis of this distinction would probably require a prohibitive amount of labor.

¹ Note, however, the effect of East and Hayes's addition to this theory, discussed below.

The view that vigor depends upon the heterozygosis of the individual seems to me inherently more probable than that it is due to the presence of certain dominant genes. The former view admits of a plausible foundation in cell physiology, and the essence of it may be extended to cases of decrease of vigor in which there is no change in the genotypic constitution, and which are therefore without the pale of either theory.

LOSS OF VIGOR NOT ACCOUNTED FOR BY THE MENDELIAN EXPLANATIONS.

It has been shown in a former paper (Shull, 1912) that parthenogenetic lines of *Hydatina* may, and usually do, become less vigorous as parthenogenesis proceeds. This conclusion has been confirmed by Whitney (1912). The same phenomenon, though disputed by Woltereck (1911), has been reported in *Cladocera* by Papanicolau (1910). Some workers have found that clones of *Paramecium* decrease in vigor with long continued fission, though Woodruff (1911) has shown that this phenomenon is not universal. In none of these cases, so far as known, is there any change in the genotypic constitution throughout the line; hence a change from heterozygosis to homozygosity is not responsible for the decrease of vigor. The loss of vigor usually spoken of as senescence likewise occurs without, so far as known, any change in zygotic constitution.

The physiological explanation which I am about to offer includes the view that heterozygosity determines vigor, and covers also the cases of parthenogenetic lines and clones just mentioned, and perhaps also of senescence.

A PHYSIOLOGICAL EXPLANATION OF VIGOR.

Vigor may be thought of as dependent on the rate of metabolism. Lillie (1912), in his studies of fertilization, concludes that the increased metabolism that accompanies the development of the egg is due to an accelerated interaction between nucleus and cytoplasm. The introduction of new nuclear elements into the cytoplasm of the egg, which occurs in cross fertilization, may be supposed to disturb the equilibrium, create a greater reaction between nucleus and cytoplasm, thereby increasing metabolism, and hence vigor. On this view, it is not the fact

that the constitution of the F_1 individuals is Mm that makes them vigorous, but the interaction of a nucleus¹ of constitution Mm with a mass of cytoplasm accustomed, so to speak, to a nucleus of constitution MM or mm . If it were possible to remove from an egg its own nucleus, and substitute for it a nucleus slightly different, but not so different as to be "incompatible," with a diploid set of chromosomes, and have it develop normally, it should, on my view, produce an individual more vigorous than its parent, even if the introduced nucleus were completely homozygous. On this view, a line that has become homozygous need not have reached its minimum of vigor, as it must on both of the other views discussed.

In animals that reproduce by parthenogenesis or fission, the long continued interaction between cytoplasm and nuclei that suffer no change of genotypic constitution, may bring about an approach to equilibrium, thereby decreasing metabolism, and hence vigor.

In like manner, continued production of somatic cells without change of genotypic constitution in the nucleus may cause an approach to equilibrium resulting in senescence in the metazoan individual. The cases in which a high standard of vigor is maintained notwithstanding inbreeding, as in wheat and tobacco, or in the absence of genotypic change, as in Woodruff's paramaecia, are not so easily explained. They may be due to any one of several causes. If metabolism be maintained by a reversible reaction between nucleus and cytoplasm, vigor could be sustained indefinitely. Or the interaction may be kept up by changes in the cytoplasm, changes due to variable nutrition or other external agents. These are mere suggestions.

East and Hayes have suggested a physiological foundation for the heterozygosis view. They hold that increased vigor in hybrids is due to more rapid cell division, and that the stimulus to this more rapid division is given by the presence of genes in the heterozygous condition. To me it seems that the stimulus is due, not to any effect that the two parental contributions to the nucleus may have directly upon one another, but to the effect

¹ It is assumed without argument that the representatives of body characters reside in the chromosomes.

of a changed nucleus and a (relatively) unaltered cytoplasm upon each other.

Perhaps this suggestion must remain in part mere speculation; but the science of cell physiology is still young, and much may be discovered that will make the proposed view either probable or improbable.

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